REMARKS

The Office Action of April 5, 2007 presents the examination of claims 1-20; these claims remain pending. Minor amendments are made to claims 5-7 to capitalize the font for the term ALEXA FLUOR to reflect the trademark nature of the term. Claim 18 is amended as to dependency. Both amendments were recommended by the Examiner.

Objections to the specification and claims

The specification is objected to as failing to present the trademark ALEXA FLUOR in all capital letters. A substitute specification is provided attached hereto that so presents this trademark. Both a "clean copy" and a "track changes" copy of the substitute specification are provided for the Examiner's review.

The present claims are also amended to present the trademark ALEXA FLUOR in capital letters.

No new matter is added by any of these amendments.

The Examiner also objects to the incorporation by reference of several items of the periodical literature at the end of the specification. The Examiner's objection is noted, but at this time Applicants are not relying upon any material incorporated by reference to overcome any ground of rejection, and so further amendment of the specification is not necessary at present.

Claim 18 is objected to as failing to further limit claim 17 from which it depends. Claim 18 is amended to be dependent from claim 16 as suggested by the Examiner. Applicants submit that this amendment also resolves the issue of substantial duplication of claims 17 and 18.

Rejection under 35 USC § 112, second paragraph

Claims 5-7 and 11-13 are rejected under 35 USC § 112, second paragraph, as allegedly being unclear in the description of a fluorophore as "ALEXA FLUOR 594". This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

The Examiner asserts that ALEXA FLUOR is a trade name or trademark that identifies only the source of the fluorophore and does not describe it. However, ALEXA FLUOR 594 is a specific molecule, and discussion with technical representatives of Molecular Probes (owner of the trademark) revealed that no IUPAC name has been given to the dye series nor has it been registered with CAS. The ALEXA FLUOR dyes are generically sulfonated rhodamines. A conjugate of ALEXA FLUOR 594 to a protein is illustrated in the attached Exhibit 1 for the record.

Rejections over prior art

Over Thompson and Tsien

Claims 1-3, 8-9, 14-18 and 20 are rejected under 35 USC § 103(a) as being unpatentable over Thompson '236 in view of Tsien '797. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

Applicants submit that the Examiner fails to establish *prima facie* obviousness of the invention as claimed. In particular, the combination of the references cited fails to disclose or suggest each and every feature recited in the claims.

The present invention, as recited in claim 1 and claims dependent thereon, recites that a ratio of fluorescence of an acceptor fluorophore after excitation of a donor-acceptor system at a first wavelength for donor excitation and at a second wavelength for acceptor excitation is determined. Such an excitation ratiometric method is not described or suggested in either of Thompson '236 or in Tsien '797.

Thompson '236 teaches determination of an analyte, e.g. zinc ion, using a fluorescently-labeled variant of apocarbonic anhydrase (i.e. CA lacking a bound metal ion) and a chromophoric ligand whose binding to carbonic anhydrase is promoted by binding of zinc to the apoprotein; the label and chromophoric ligand are chosen such that the fluorescent label's emission overlaps the chromophoric ligand's absorbance and the ligand can act as energy transfer acceptor to the label acting as donor. That is, the chromophoric ligand acts as a quencher of emission of the fluorescent label when the two are in proximity, i.e. upon zinc binding to the apocarbonic anhydrase. Thompson '236 teaches that the analyte is determined by measuring changes in energy transfer by changes in donor intensity or lifetime; Thompson does not teach (as the Examiner points out in 8.) that the ligand bound to the CA is fluorescent and can serve as a donor. Thompson '236 does not teach a ratio of fluorescence intensities, and is unworkable as a ratio.

The combination with Tsien '797 does not remedy the deficiency of Thompson '236 of failing to disclose analysis of the ratio of fluorescence at two different excitation wavelengths.

Tsien '797 teaches determination of an analyte based on the analyte-induced dissociation of two polypeptide subunits, the subunits having been labeled with fluorescent labels D and A chosen such that the fluorescence emission of D overlaps significantly with the absorbance of A, and there is an increase in energy transfer from D to A upon dissociation of the analyte causing the subunits to be brought together. Tsien teaches that the energy transfer may be measured by (Col. 6, lines 39 to 49) measurement of the ratio of emission at the wavelengths of emission of D and A (i.e. an emission ratiometric method), or by the fluorescence lifetime of D, or by the rate of photobleaching of D. Tsien '797, at lines 39-40 of column 6, err in saying that their invention is workable by "providing energy near the excitation wavelength of D or A;" , because exciting at the excitation wavelength of the acceptor fails to provide any excitation for the donor, and thus there would be no analyte-dependent change in the ratio of emissions at the wavelengths of emission of D and A, nor change in the lifetime of D, nor in the photobleaching of D. To the degree that Tsien '797 would be understood as suggesting measurement using excitation at the excitation wavelegths of both D and A, their invention would be inoperable if the donor is not both excited and its emission observed. That is, Tsien '797 requires that emission from the D fluorophore be observed, and the present invention is such that this is not done. Tsien '797 particularly does not teach or suggest determining the analyte by measuring the ratio of intensities at the acceptor emission wavelength when excited at the donor and acceptor excitation wavelengths (an "excitation ratiometric" determination), as the present application teaches, and there is no evidence in the reference that such an approach is even workable with their invention.

Indeed, to the contrary, the Tsien '797 reference includes at least three items of evidence that their invention is not workable as an excitation ratiometric determination. First, energy

transfer between donor and acceptor in the large, multipolypeptide complex is evidently of only middling efficiency, despite the excellent spectral overlap of the donors and acceptors chosen, as can be seen by the substantial intensity at the donor emission wavelength of 515 nm even in the absence of analyte (Figure 2 of Tsien, et al.). Second, with the donor-acceptor pairs disclosed by Tsien, there is substantial emission from the donor (unbound) present at the acceptor emission wavelength. Third, for an excitation ratiometric approach in this case it is also desirable that the absorption of the acceptor at the donor excitation wavelength be as poor as possible. However, in the examples disclosed by Tsien '797 this is not the case.

These three aspects of the Tsien '797 reference combine so that the difference in intensity at the acceptor emission wavelength between the donor when bound and free is very small, and thus the ratio changes negligibly. On the other hand, for an effective excitation ratiometric approach based on energy transfer, it is desirable that the energy transfer efficiency be as large as possible, that the unbound donor should emit negligibly at the emission wavelength of the acceptor, and the acceptor should absorb weakly at the donor excitation wavelength. Tsien '797 does not describe any of these three factors.

Thus, it is plain that the combination of Thompson '236 with Tsien '797 fails to disclose several aspects of the present invention, and principally that an excitation ratiometric approach to determination of the analyte should be taken. Therefore, the presently-claimed invention as described in claims 1-3, 8-9, 14-18 and 20 is not *prima facie* obvious in view of the combination of these two references and the instant rejection should be withdrawn.

Over Thompson in view of Tsien and further in view of Jensen

Claims 14-18 and 20 are rejected under 35 USC § 103(a) as being unpatentable over Thompson '236 in view of Tsien '797 and further in view of Jensen. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

Claims 14-18 and 20 describe an excitation ratiometric method for determining an analyte in the same manner as claim 1. However, in the method of claims 14-18 and 20, the fluorophore labeled protein is a CA that is conjugated to a fluorescent protein. The combination of Thompson '236 with Tsien '797 fails to establish *prima facie* obviousness of an excitation ratiometric approach to determination of an analyte for the reasons explained above.

Jensen does not remedy these deficiencies. The Examiner cites Jensen merely for its disclosure of use of an engineered green fluorescent protein (GFP) in a fluorescence energy transfer (FRET) method. Jensen does not, however, contribute any teaching that an excitation ratiometric analysis should be used for determining the amount of any analyte. Thus, the combination of Thompson '236 with Tsien '797 and Jensen still fails to disclose or suggest that an analyte should be determined using an excitation ratiometric method.

Accordingly, claims 14-18 and 20 are not *prima facie* obvious over this combination of references and the instant rejection should be withdrawn.

Over Thompson '236 in view of Tsien '797 and Thompson '99

Claims 4, 10 and 19 are rejected under 35 USC § 35 USC § 103(a) as being unpatentable over Thompson '236 in view of Tsien '797 and Thompson, SPIE 1999, vol. 3603). This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

Applicants submit that the Examiner fails to establish *prima facie* obviousness of the claimed invention. Again, the combined references fail to disclose or suggest each and every recitation in the claims.

Claim 4 depends from claim 1 and further describes the donor fluorophore. Claim 19 is dependent from claim 16 and describes the donor fluorophore in the manner of claim 4. Claim 10 is dependent from claim 1 and further describes the carbonic anhydrase enzyme.

As explained above with respect to claims 1 and 16, the combination of Thompson '236 with Tsien '797 fails to establish *prima facie* obviousness of an excitation ratiometric approach to determination of an analyte. Thompson '99 does not remedy this deficiency of the combination of Thompson '236 and Tsien '797 to describe or suggest the basic invention. Thompson '99 discloses a method utilizing anisotropy of fluorescence polarization to determine binding of an analyte to CA (see, page 16, line 13); the reference does not at all suggest that measurement at two different wavelengths, one exciting a donor molecule and one exciting an acceptor molecule directly, should be used. Therefore, adding Thompson '99 to the combination of Thompson '236 and Tsien '797 still provides a set of references that together fail to disclose or suggest an excitation ratiometric approach to measuring an analyte.

Accordingly, the combined references fail to establish *prima facie* obviousness of the presently-claimed invention, and so the instant rejection should be withdrawn.

In view of the above amendment, applicants believe the pending application is in condition for allowance. The favorable actions of withdrawal of the standing rejections and allowance of the claims are requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Dr. Mark J. Nuell Reg. No. 36,623 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

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Respectfully submitted,

By /mjn/
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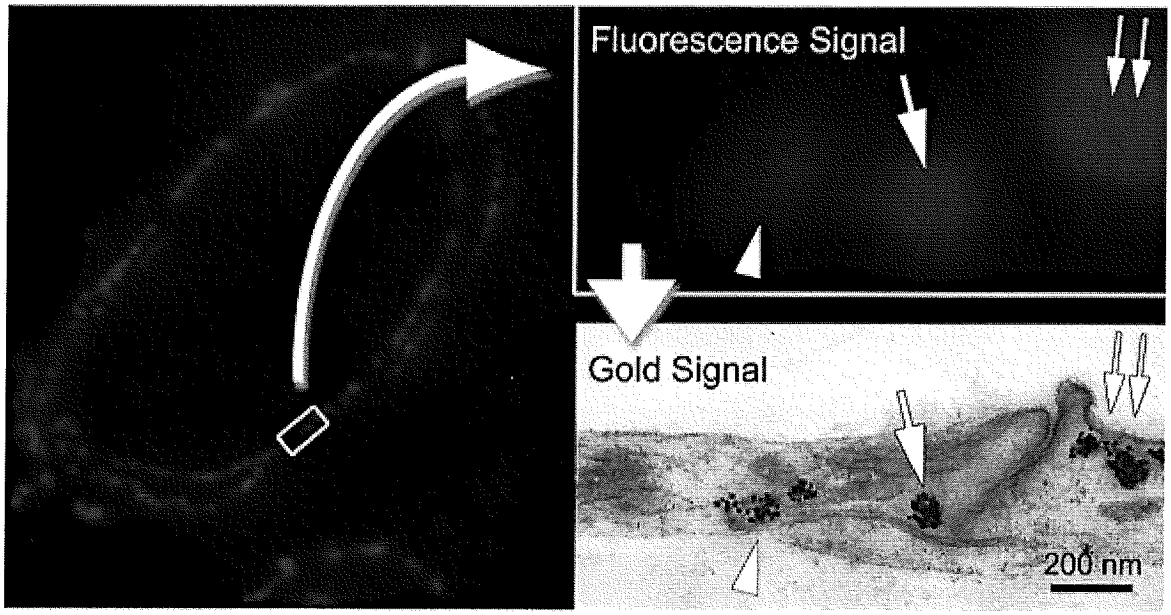
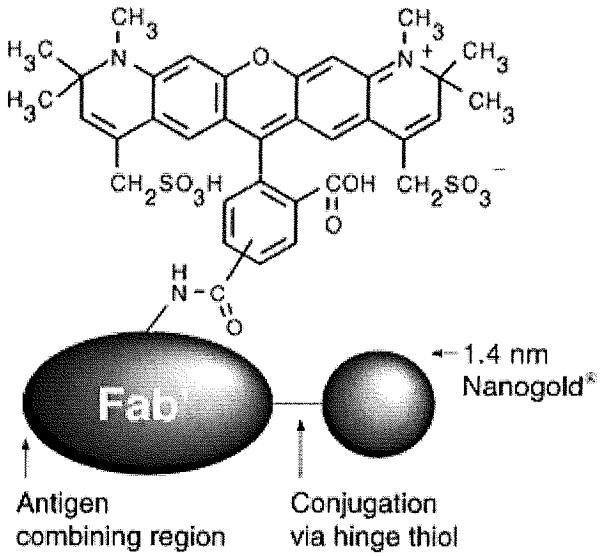
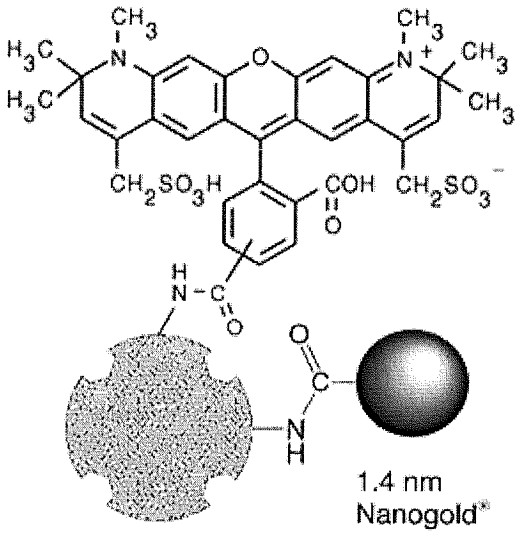
Attachments: Substitute specification clean copy and track-changes copy
Exhibit 1 – from Invitrogen/Molecular Probes webpage

Alexa Fluor® 594 FluoroNanogold Staining of Caveolin-1a



Localization of caveolin-1a in ultrathin cryosection of human placenta using a new FNG; caveolin 1 alpha is primarily located to caveolae in placental endothelial cells. One-to-one correspondence is found between fluorescent spots and caveola labeled with gold particles (right). Ultrathin cryosections collected on formvar film-coated nickel EM grids were incubated with chicken anti-human caveolin-1a IgY for 30 min at 37°C, then with biotinylated goat anti-chicken F(ab)$_2$ (13 mg/ml) for 30 min at 37°C, then stained with ALEXA-594 FluoroNanogold-Streptavidin (1:50 dilution) for 30 min at room temperature. Non-specific sites on cryosections were blocked with 1% milk - 5% fetal bovine serum-PBS for 30 minutes at room temperature (figure courtesy of T. Takizawa, Ohio State University, Columbus, OH).





Structure of Alexa Fluor® 594 FluoroNanogold Fab' and Streptavidin conjugates, showing covalent attachment.*